Express Mail No.: EL134447705US

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

#19
B.G.J
8/19/98

Application of: Samulski et al.

Application No.: 08/475,470 Group Art Unit: 1809

Filed: June 7, 1995 Examiner: A. Nelson

For: ADENO-ASSOCIATED VIRUS VECTOR AND CIS-ACTING REGULATORY AND PROMOTER ELEMENTS CAPABLE OF EXPRESSING AT LEAST ONE GENE AND METHOD OF USING SAME FOR GENE THERAPY

Attorney Docket No.: 7639-077 (formerly 115132-4)

DECLARATION UNDER 37 CFR §1.132 BY RICHARD J. SAMULSKI

Honorable Commissioner of Patents and Trademarks
Washington, D.C. 20231

Sir:

I, RICHARD JUDE SAMULSKI, do declare:

1. I am an inventor of the above-identified application.

2. I am an associate professor at the Department of Pharmacology at the University of North Carolina, with which I have been affiliated since June, 1993. I am experienced in the field of virology, particularly as it relates to adeno-associated virus (AAV) and the use of AAV for gene therapy. (See Curriculum Vitae, attached herewith as Exhibit A).

3. The invention disclosed in the above-identified application relates to recombinant adeno-associated virus vectors for gene delivery and regulated tissue specific expression in a host. The recombinant adeno-associated

vectors contain a mammalian gene of interest, genetically engineered adjacent to *cis*-acting regulatory and promoter elements, in such a way as to regulate expression in a tissue specific manner. The recombinant vectors can be used therapeutically to treat a variety of different genetic or acquired diseases.

4. Studies were conducted to determine whether recombinant AAV vectors could be used to efficiently transfer genes of interest in an *in vivo* setting. In particular, recombinant AAV vectors were used to transduce enriched primate hematopoietic cells which were then transplanted back into the primate host. The recombinant AAV vectors were genetically engineered to contain the neo^r gene.

5. Primate blood derived cells were harvested and the progenitor cells were enriched for by positive immunoselection for cells expressing the CD34+ antigen. 1-2 x 10^7 immunoselected cells from six animals were transduced with recombinant AAV virus for 6-8 hours in media, with or without, stem cell factor and interleukin-6 (CSF+/CSF-). Following incubation with recombinant AAV virus, the cells were washed and transplanted back into γ-irradiated primates.

6. Detection of transduced viral DNA in the transplanted primates was performed using a semi-quantitative PCR assay for detection of the neo^r gene. As indicated in FIG. 1, viral DNA was detected in peripheral blood mononuclear cells (PB) and bone marrow (BM) from three of the six animals.

7. Viral DNA from the PB and BM of one animal (J352) was PCR positive at day 76. The animal remained positive until day 128 when it developed a bacterial infection and was euthanized. All the hematopoietic organs (liver, spleen, bone marrow, and thymus) taken from the animal at the time of death were positive for the presence of the transgene. Non-hematopoietic organs such as the brain, skeletal muscle and kidney were PCR negative for the transgene. The presence of the transgene was detectable in both flow cytometric purified populations of granulocyte and lymphocyte lineages as indicated in FIG.2.

8. Blood derived cells from the PCR postive animal (J352) was tested for expression of the transduced neomycin gene. Blood derived cells were harvested from the animal and plated in methylcellulose in the presence or absence of G418 (1.0 mg/ml). As indicated in FIG. 3, G418-resistant colonies were obtained from the PCR positive animal (J352), while no colonies were obtained from the control animal. The data strongly supports that rAAV not only infects primary hematopoietic cells, but also, expresses a functional protein.

9. The data presented indicates normal hematopoietic reconstitution of lethally irradiated primates following transplantation with recombinant AAV transduced CD34+ cells. In addition, it was observed that recombinant AAV transduction levels can persist for up to at least three months (the duration of the experiment). *In vitro* colony forming unit (CFU) assays performed on the transplanted animals showed

normal marrow reconstitution of neutrophils, erythrocytes, and platelets comparable to control animals indicating that transduction of recombinant AAV into blood derived cells does not adversely affect reconstitution. The detection of the virally transferred transgene (neo^r) in both myeloid and lymphoid lineages further indicates the successful transduction of progenitor cells by recombinant AAV. Additionally, the ability of transduced cells to grow in the presence of G418 indicates successful expression of a functional protein.

10. I hereby declare further that all statement made herein by my own knowledge are true and that all statements made on information and belief are believed to be true and further that I make these statement with the knowledge that willful false statements and the like are punishable by fine or imprisonment, or both, under §1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application of any patent issuing thereon.

Date 8/11/98

Richard J. Samulski, Ph.D.

FIG. 1

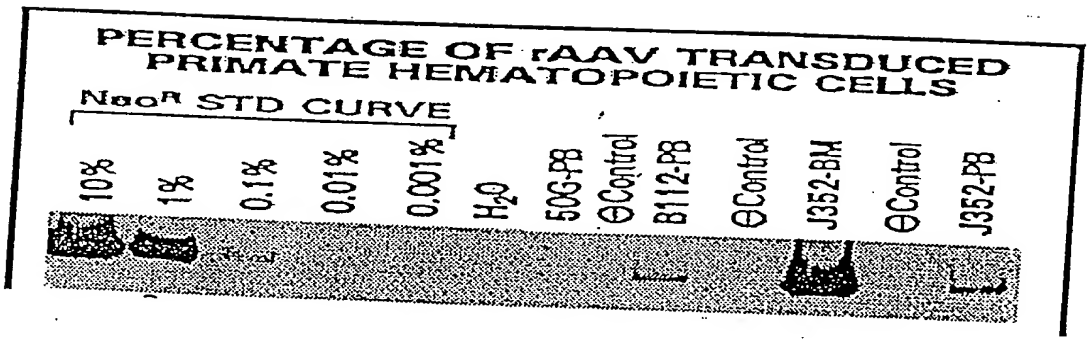
PERCENTAGE OF rAAV TRANSDUCED PRIMATE HEMATOPOIETIC CELLS
Neo^R STD CURVE
10%
1%
0.1%
0.01%
0.001%
H2O
506-PB
Θ Control
B112-PB
Θ Control
J352-BM
Θ Control
J352-PB

FIG. 2

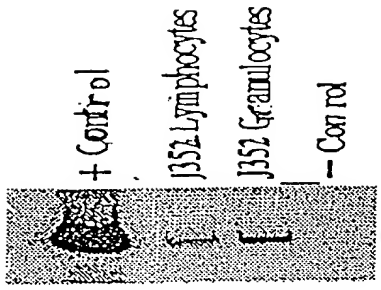

FIG. 3

Animal #I352	# of colonies/5x105 cells
-G418	60
+G418	10
Control Animal	
-G418	40
+G418	0

CURRICULUM VITAE
Richard Jude Samulski, Ph.D.

PERSONAL

Date & Place of Birth:	March 10, 1954, Augusta, Georgia
Marital Status:	Married; two children
Social Security Number:	257-02-1643
Work Address:	Gene Therapy Center 7119 Thurston Bowles Bldg., CB# 7352 School of Medicine University of North Carolina Chapel Hill, NC 27599-7352 (919) 962-3285
Home Address:	102 Darlin Circle Chapel Hill, NC 27514 (919) 933-7210

EDUCATION

1972-1976	B.S., Microbiology Clemson University, Clemson, SC
1978-1982	Ph.D., Molecular Biology Laboratory of Dr. Nicholas Muzyczka, University of Florida, Gainesville, FL

PROFESSIONAL EXPERIENCE

6/1993 - present	Associate Professor

EXHIBIT A
SAMULSKI DECLARATION
Applin No. 08/475,470
June 7, 1995

1984-1986	Postdoctoral Research Fellow Laboratory of Dr. Thomas Shenk, Princeton University, Princeton, NJ
1982-1984	Postdoctoral Research Fellow Laboratory of Dr. Thomas Shenk, Microbiology Department, SUNY, Stony Brook

ACADEMIC AND PROFESSIONAL HONORS

South Florida Branch ASM Student Research Award, First place, 1981
Medical Guild Graduate Student Research Award, Third place, 1982
NIH Pre doctoral Trainee, 1981-1982
NIH Postdoctoral Fellow, 1983-1986
Outstanding Young Men of America Award, 1990
University of Pittsburgh, President's Distinguished Research Award, 1991
American Heart Association Established Investigator 1991-1996
William C. Friday Fellowship for Human Relations nominee. 1995.

PROFESSIONAL ACTIVITIES

Pittsburgh Area Recombinant DNA Committee, 1990
Advisor for Minority Research Apprentices Program, Summer Semester 1989 & 1990
Pittsburgh Cancer Institute, member, September 1987-July 1993
University of North Carolina Lineberger Comprehensive Cancer Center, member, August 1993-current
NIH/ADAMHA Consultant, 1990-current
NIH Study Section, Virology, 10/92
Member, Mammalian Genome Society 1992
University of Pittsburgh Technology Transfer Committee, 1992
Ad hoc reviewer for National Science Foundation grant applications
Ad hoc reviewer for March of Dimes grant applications
University of North Carolina Curriculum of Genetics, member, 1993-present
UNC Center for Gastrointestinal Biology and Disease, member, 1993-present
Member, International Advisory Committee for the Xth International Congress of Virology
Participation in Gene Therapy Center Video for Health Sciences Media, Todd Wilson, Producer, April 1994

INVITED SEMINARS

University of Pittsburgh, Public Health, March 1987
SUNY at Buffalo, Department of Microbiology, April 1987
Emory University, October 1988
Bethesda Research Laboratories, October 1988
Cornell University, January 1989
University of Pittsburgh, Medical School, April 1989
Carnegie Mellon University, Department of Microbiology, April 1989
Ciba-Geigy, Basel, Switzerland, December 1989
Institute of Virus Research, German Cancer Research Center, Heidelberg, Germany, December 1989
Institute of Virology, Koln, Germany, December 1989
Duke University Medical Center, January 1990
University of Pittsburgh, Public Health, January 1990
University of Pittsburgh, Public Health, March 1990
SUNY, Stony Brook, Department of Microbiology, April 1990
St. Louis University Medical School, June 1990
University of Pittsburgh, Human Genetics, October 1990
University of Pittsburgh, G.S.P.H., February 1991
Baylor College of Medicine, Molecular Genetics Department, March 1991
University of Washington, Markey Molecular Medicine Center, March 1991
University of Pittsburgh, Department of Microbiology & Immunology, May 1991
Indiana University, Department of Microbiology, May 1991
University of Pittsburgh, School of Medicine, June 1991
University of Michigan, Howard Hughes Medical Institute, August 1991
Case Western Reserve University, Dept. of Physiology & Biophysics, October 1991
University of North Carolina at Chapel Hill, School of Medicine, January 1992
Academic Affairs Committee, University of Pittsburgh, February 1991
Merck Cancer Research Center, Philadelphia, PA, September 1991
Children's Hospital of Pittsburgh, Lung Group, March 1992
The Cleveland Clinic Foundation, Cleveland, OH, April 1992
University of Illinois at Chicago, College of Medicine, May 1992
Amgen, Thousand Oaks, CA, August 1992
Children's Hospital of Pittsburgh, Grand Rounds, February 1993
Virginia Tech, Department of Biology, Blacksburg, VA, February 1993
Keystone Symposia Meeting M, Lake Tahoe, CA, March 16, 1993

University of North Carolina at Chapel Hill, Curriculum in Toxicology Monthly Graduate Student Lecture, "Current Research in Gene Therapy. January 13, 1995.
University of Florida, College of Medicine, Gene Therapy Center. "Gene Therapy for Parkinson's - One Step Away From the Clinic"., February 1, 1995.
University of Florida, College of Medicine, Gene Therapy Center. "Gene Therapy in Parkinson's Disease: The Data", February 1, 1995.
University of North Carolina at Chapel Hill, School of Medicine, Gene Therapy in the Brain Workshop. Presenter and moderator. March 3, 1995.
National Eye Institute, Laboratory of Immunology. March 7, 1995.
North Carolina Biotechnology Center. June 13, 1995.
Advanced Lecture on Gene Therapy. Istituto Di Technologie Biomediche Avanzate, Varenna, Italy, October 8, 1995.
Center for Advanced Biotechnology and Medicine, Symposium: "Therapeutic Applications of Viruses". Berkeley, California, November 21, 1995
National Institute of Diabetes and Digestive and Kidney Diseases. Workshop on "AAV Vectors: Gene Transfer into Quiescent Cells". Workshop Chairperson. December 6, 1995.
National Institute of Diabetes and Digestive and Kidney Diseases. Workshop on "AAV Vectors: Gene Transfer into Quiescent Cells". Speaker "AAV Vector Transduction in Muscle and Brain", December 6, 1995.
Ohio State University Biochemistry Program Seminar Series, Columbus, Ohio, November 7, 1995.
University of North Carolina at Chapel Hill, Neurobiology Spring Semester Colloquium. "In Vivo Gene Delivery for the Study of Neurological Disorders". February 16, 1996.
Duke Institute for Learning in Retirement and the Museum of Life and Science, lecture as part of course on Science and Technology, Duke University September 11, 1996.
National Institute of Health, invited speaker, RTP, October 4, 1996.
Cold Spring Harbor Labortory, Gene Therapy Conference, invited speaker, Cold Spring Harbor, New York, September 25-29, 1996.
James Wilson Laboratory, invited speaker on "AAV Vectors for Brain Delivery." University of Pennsylvania Health System. October 27-29, 1996.
European Working Group on Human Gene Transfer and Therapy, invited speaker on "AAV Vectors for Brain Delivery," Netherlands Institute for Brain Research, Amsterdam, The Nethetherlands, November 14-17, 1996.
Cambridge Healthtech Institute, invited speaker in the Aritificial Self-Assembling Systems for Gene Delivery "AAV Vectors in Gene Therapy," Coronado, California, November

Cystic Fibrosis Foundation, Williamsburg Conference, Gene Therapy Section, Williamsburg, VA, Invited Speaker, "Adeno-associated Virus." June 1-4, 1997.
International Symposium on Gene Therapy and Hemophilia, University of North Carolina, North Carolina, Co-Chairman and speaker, "AAV Vectors for Hemophilia.." September 4-6, 1997.

Plenary Lectures

American Society of Virology, *Parvovirus Meeting*, July 1987
Kibbutz Ma'ale Hachamisha, Israel, *International Meeting on Parvovirus*, November 1989
Gordon Conference on Human Genetics, July 1991
EMBO Workshop: *Molecular Biology of Parvoviruses*, Copenhagen, August 1991
Sandoz, *HIV: Human Therapy Conference*, Vienna, October 1991
NIH/NCDDG, *Treatment of HIV Infections*, San Diego, CA, November 1991
Cystic Fibrosis Gene Therapy Workshop, Westfields, VA November 1991
NIH Conference on Human Gene Therapy, Washington, D.C., December 1991
4th Annual Conf. on Hemoglobin Switching, Rosario, WA, May 1992
Cystic Fibrosis Conference, Virginia, June 1992
March of Dimes Clinical Genetics Conference, Stanford, CA, July 1992
Banbury Conference, Cold Spring Harbor, NY, July 1992
Gene Therapy Conference, Cold Spring Harbor, NY, September 1992
6th Annual North American Cystic Fibrosis Conf., Washington, D.C., October 1992
USC/Amgen Symposium *Gene Therapy for Human Disease*, Pasadena, CA, November 1992
NIDDK *Treatment of Genetic Diseases*, Bethesda, MD, November 1992
NCDDG *Ribozymes/Gene Therapy in the Treatment of HIV*, San Diego, CA December 1992
Cystic Fibrosis Gene Therapy Workshop, Tampa, FL, January 1993
American Society for Microbiology, Atlanta, GA, May 16, 1993
Cystic Fibrosis Gene Therapy Workshop, Williamsburg, VA June, 1993
AAAS *Science Innovation '93*, Boston, MA, August 7, 1993
International Symposium on *Cancer Treatment,* Nagoya, Japan, September 20, 1993
American Society of Human Genetics 43rd Annual Meeting, October 1993.
5th Parvovirus Workshop, November 1993
AASLD 44th Annual Meeting Research Workshop *The Prospect of Gene Therapy for Liver Diseases* November 1993

Falk Symposia, Basel Switzerland, October 17-24, 1995.
III Congreso de la Sociedad de Medicina Interna de Arago, Pamplona, Spain, October 26, 1995.
1996 Spring Meeting of the Association of Clinical Scientists. Opening Session Presentation, May 2, 1996.
Keystone Symposia, Taos, New Mexico. February 4-10, 1996
American Society of Neurochemistry's Sattellite Symposium on Gene Therapy for Neurological Disorders. March 2, 1996.
International Centre for Genetic Engineering and Biotechnology and the Universities of Di Padova and Loma Linda. Trieste, Italy, April 11-14, 1996.

DEPARTMENTAL ACTIVITIES

Faculty Search Committee 1988
Chair, Faculty Search Committee 1992
Pittsburgh Area Recombinant DNA Committee
Mellon Fellowship Committee, 1989
Graduate Recruiting Committee, 1990
University Selection Committee for the Burroughs Wellcome Foundation Career Awards in the Biomedical Field
Faculty Search Committee 1996

Thesis Committees:
Brenda Hall
Susan Spence
Hui Chen
Anthony Youlton
Fran Sverdrup
Scott Erme
Beverly Hamilton
Uma Chandran
Xiaojun Cheng
Forrest Ferrari
Xiaohong Gu
Deanna Hapke
Weidong Xiao
Marilee Shelton

First year graduate students, Biological Sciences.
1989, 1990, 1991, 1994, 1995

Undergraduate Directed Research:
Roger Williams
Hengameh Bazmi
Renay Oshop
Joseph Molitierno

Laboratory Members

GRADUATES
Lynne Hunter, Ph.D. 1988- 1992; B.S., Syracuse University, NY
Xiaodong Zhu, Ph.D. student 1987 - 1993: M.S., Nankai University, China

PRE DOCTORAL TRAINEES:
Alison Slinsky, BS, student 1992 - 1993, B.S., Allegheny College
Forrest Ferrari, BS, student 1991 - 1997; B.S., Univ. of Pittsburgh, PA, 1991. PhD UNC-CH, 1997.
Weidong Xiao, BS, student 1992 - 1996, B.S., Xiangtan University, China, 1992. PhD UNC-CH 1996.
Candace Summerford,, BS. student 1995-present, B.S., Fitchburg State College, Fitchburg, MA
Terry Amiss, BS, MS, student 1996-present, BS Purdue 1983, MS Wright State 1985.
Rebecca Haberman, BS, student 1996-present. BS Duke Universsity 1992.

POSTDOCTORAL TRAINEES:
Qichang Shen, 1988 - August 1989; Ph.D., Shanghai Institute of Biochemistry, China. Currently: Research Associate, University of Massachusetts Medical Center.
Xiao Xiao, 1989-1992, PhD, University of Pittsburgh.
Gina Trimbur, 1992-1993, PhD. University of Pittsburgh
Fabienne Rolling, 1992-1995, Ph.D. University of Aix-Marseille II
David Ansardi, 1994 - 1996, Ph.D., University of Alabama at Birmingham
Jeffrey Bartlett, 1993 - 1997, Ph.D., University of Pittsburgh School of Medicine
Anna Skulimowski, 1993 - 1996; Ph.D.John Curtin School of Medical Research, Australian National University, Canberra
Charlie Yang, 1994-Current, Brandeis University, Wltham, MA
Gabriele Kroener-Lux, 1995-current, PhD, Institution for Molecular Biololgy Medicine, Essen.

Xilin Li
Kailing Fu

AWARDS TO MEMBERS OF THE LABORATORY

Lynne Hunter
Pre-Clinical Fellowship, Sandoz Pharmaceutical Company; Basel, Switzerland
June - August 1990.
Ben Franklin Fellowship, 1989.
Joseph Molitierno
Howard Hughes Undergraduate Research, 5/92
Candace Summerford
Hoechst-Celanese Corporation/UNC-CH 1995 Excellence Award, December 1995

TEACHING EXPERIENCE

Bacterial Genetics, B.S. 1280, University of Pittsburgh, Winter 1987.
Graduate Student Seminar, B.S. 2450, University of Pittsburgh, Fall 1988 & 1991.
Virology, University of Pittsburgh, B.S. 1730, Winters 1988 - 1991.
Graduate Virology Course; Lecturer, G.S.P.H., University of Pittsburgh, Winters, 1989 - 1992
Graduate Core Course, University of Pittsburgh, B.S. "MCDB", Virus Module, Fall 1991, 1992.
Virology, University of Pittsburgh, B.S. 1730, Winter 1993.
Gene Therapy and Cancer, Biochemistry 105. Undergraduate class, December 5, 1994
Gene Therapy using Parvovirus, Graduate Students, Virology (Microbiology & Immunology 130), December 7, 1994. .
Molecular Biology Undergraduate Course (Biotechnology and It's Social Impact) - "Gene Therapy-Practice and Caveats". Princeton University, February 7, 1995.
Pharmacology, UNC-CH, Course Director, Graduate Course. Tutorial in Pharmacology,"Techniques in Gene Therapy" 221. Spring 1995. 12 Students.
Pharmacology, UNC-CH, Lecture for Pharmacology # 205, The Molecular Pharmacology of Cancer., 11/15/95
Microbiology, UNC-CH, Lecture for Microbiology # 130, Molecular Virology, 11/28/95
Biochemistry, UNC-CH, Lecture for Biochemistry # 105, Molecular Biology, 11/29/95
Neurobiology Curriculum Speakers Series, UNC-CH, Lecture, 2/16/96

2. Samulski, R.J., Berns, K., Tan, M., and Muzyczka, N. . Cloning of AAV into pBR322: Rescue of intact viruses from the recombinant plasmid in human cells. P. N. A. S. USA, 79: 2077-2081, 1982.

3. Samulski, R.J., Srisvastava, A., Berns, K., and Muzyczka, N. Rescue of adeno-associated virus from recombinant plasmids: Gene correction within the terminal repeats of AAV. Cell, 33: 135-143, 1983.

4. Hearing, P., Samulski, R.J., Wishart, W.L., and Shenk, T. (1987).Identification of a repeated sequence element required for efficient encapsidation of the adenovirus Type 5 chromosome. J. Virol., 61: 2555-2558, 1987.

5. Samulski, R.J., Chang, L.S., and Shenk, T. (1987). A recombinant plasmid from which an infectious adeno-associated virus genome can be excised *in vitro* and its use to study viral replication. J. Virol., 61: 3096-3101, 1987.

6. Samulski, R.J. and Shenk, T. (1988). Adenovirus EIB-55K polypeptide facilitates timely cytoplasmic accumulation of adeno-associated virus mRNAs. J. Virol., 62: 206-210, 1988.

7. Samulski, R.J., Chang, L.S., and Shenk, T. Helper-free stocks of recombinant adeno-associated viruses: Normal integration does not require viral gene expression. J. Virol., 63: 3822-3828, 1989.

8. Srivastava, C.H., Samulski, R.J., Lu, L., Larsen, S.H., Srivastava, A. (1989) Construction of a recombinant human parvovirus B19: Adeno-associated virus 2 DNA inverted terminal repeats are functional in an AAV-B19 recombinant virus., P. N. A. S. 86: 8078-8082, 1989.

9. Snyder, R. Samulski, R.J. and Muzyczka, N.(1990) *In vitro* resolution of covalently joined AAV chromosomes., Cell 60: 105-113, 1990.

10. Kotin, R., Siniscalco, M., Samulski R.J., Zhu, X., Hunter, L., Laughlin, C., McLaughlin, S., Muzyczka, N., Rocchi, M., and Berns, K. Site-specific integration by adeno-associated virus., Proc Nat Acad Sci USA 87: 2211-2215, 1990.

11. Samulski, R.J. Zhu, X. Xiao, X. Brook, J.D. Housman, D.E. Epstein, N. Hunter, L.

16. Snyder, R. O., Im, D-S., Ni, T., Xiao, X., Samulski, R. J., and Muzyczka, N. Features of the adeno-associated virus origin involved in substrate recognition by the viral rep protein. J. Virol., 67, 6096-6104, 1993.

17. Miller, J.L., Walsh, C.E., Ney, P.A., Samulski, R.J., and Nienhuis, A.W. . Single copy transduction and expression of human g-globin in K562 erythroleukemia cells using recombinant adeno-associated virus vectors: The effect of mutations in NF-E2 and GATA-1 binding motifs within the HS2 enhancer. Blood, Vol. 82, 6:1900-1906, 1993.

18. Wei JF, Wei FS, Samulski RJ, and Barranger JA. Expression of the human glucoerebrosidase and arylsulfatase A genes in murine and patient primary fibroblasts transduced by an adeno-associated virus vector. Gene Therapy, Vol. 1, 4:261-268, 1994.

19. Goodman S, Xiao X, Donahue RE, Moulton A, Miller J, Walsh C, Young NS, Samulski RJ, and Nienhuis AW. Recombinant adeno-associated virus mediated gene transfer into hematopoietic progenitor cells. Blood Vol. 84, 5:1492-1500, 1994.

20. Walsh C, Nienhuis AW, Samulski RJ, Brown MG, Miller JL, Young NS, and Liu JM. Phenotypic correction of Fanconi anemia in human hematopoietic cells with a recombinant adeno-associated virus vector. J of Clinical Invest, 94:1440-1448, 1994.

21. Kaplitt MG, Leone P, Samulski RJ, Xiao X, Pfaff DW, O'Malley KL, and During MJ. Long-term gene expression and phenotypic correction using adeno-associated virus vectors in the mammalian brain. Nature Genetics, 8:148-154, 1994.

22. Miller JL, Donahue RE, Sellers SE, Samulski RJ, Young NS, and Nienhuis AW. Recombinant adeno-associated virus (rAAV) mediated expression of a human γ-globin gene in human progenitor derived erythroid cells. PNAS 91:10183-10187, 1994.

23. Rolling F and Samulski RJ. AAV as a viral vector for human gene therapy: generation of recombinant virus. Molecular Biotechnology, 3:9-15, 1995.

24. Ferrari, FK, Samulski, T., Shenk, T. and Samulski, RJ. Second Strand Synthesis is a Rate Limiting Step for Efficient Transduction by rAAV Vectors. Journal of Virology, 70:3227-3234, 1996.

29. X. Xiao, J. Li, R. J. Samulski, Efficient Long-Term Gene Transfer Into Muscle Tissue of Immunocompetent Mice By Adeno-Associated Virus Vector. Journal of Virology, 11:8098-8108, 1996.

30. X. Xiao, J. Li, T. J. McCown, R.J. Samulski, Gene Transfer by Adeno-Associated Virus Vectors into the Central Nervous System. Experimental Neurology, 144: 113-124, 1997.

31. X. Xiao, T.J. McCown, J. Li, G.R. Breese. A. L. Morrow, R.J. Samulski, Adeno-Associated Virus(AAV) vector antisnse gene transfer *in vivo* decreases $GABA_A$ $\alpha 1$ containing receptors and increases inferior collicular seizure sensitivity. Brain Research, 16:33-48, 1997.

32. J. Li, R. J. Samulski, X. Xiao, Role For Highly Regulated *rep* Gene Expression In Adeno-associated Virus Vector Production. Journal of Virology, 71: 5236-5243, 1997.

NON-REFEREED PUBLICATIONS

1. McKeon, C and Samulski, RJ. Meeting Report: NIDDK Workshop on AAV Vectors: Gene Transfer into Quiescent Cells. Human Gene Therapy 7:1615-1619, 1996.

2. Ross G, Erickson R, Knorr D, Motulsky AG, Parkman R, Samulski RJ, Straus SE, and Smith BR. Gene Therapy in the United States: A Five-Year Status Report. Human Gene Therapy 7:1781-1790, 1996.

3. Xiao X, Juan L, McCown T, Samulski RJ. Gene Transfer by Adeno-Associated Virus Vectors into the Central Nervous System. Experimental Neurology 144:113-124, 1997.

BOOK CHAPTERS

1. Walsh, C.E., Liu, J.M., Miller, J.L., Nienhuis, A.W. And Samulski, R.J. Gene therapy for human hemoglobinopathies. Mini review for the Society for Experimental Biology and Medicine , 204:289-300, 1993.

2. Samulski, R.J. Adeno-associated virus (AAV): A novel viral vector for human gene therapy. In: "Ninth Nagoya International Symposium on Cancer Treatment", Excerpta

7. Bartlett, J.S., Quattrocchi, K.B., and Samulski, R.J. The Development of Adeno-Associated Virus as a Vector for Cancer Gene Therapy. In: "The Internet Book of Gene Therapy Cancer Therapeutics", R. E. Sobol and K.J. Scanlon, eds., Appleton & Lange, Stamford, Connecticut, PP 27-39, 1995.

8. Skulimowski, AW and Samulski RJ. Adeno-associated virus: Integrating vectors for human gene therapy. IN Methods in Molecular Genetics, Volume 7, Pages 3-12, 1995. Edited by Kenneth W. Adolph.

9. Bartlett, J.S., Xiao, X, and Samulski, R.J. Adeno-associated Virus Vectors for Gene Transfer. In Gene Transfer in Neuroscience: Towards Gene Therapy of Neurological Disorders. Lowenstein, P and Enquist, L, eds. John Wiley & Sons, 1996.

10. G. K.-L., R.J. Samulski, Delivery Systems for Gene Therapy: AAV, Stem Cell Biology and Gene Therapy, John Wiley & Sons, in preparation. 1997.

11. Bartlett, JS, Samulski, RJ, Li, Y, Samulski, T. Genetically Expressed Monodisperse α -Helical Polypeptides. In Proceedings of Osaka University Macromolecular Symposium, pp 159-169, Toyonaka, Osaka, Japan, 2-5 June 1995

12. Snyder , R. O. , Xiao X., Samulski, R. J. 1996. Production of Recombinant Adeno-Associated Viral Vectors. In Current Protocols in Human Genetics. 12.1.1-24. N. Dracopoli, J. Haines, B. Krof, D. Moir, C. Morton, C. Seidman, J. Seidmen, and D. Smith, eds. John Wiley and Sons Publisher, New York.

13. Bartlett, J.S., Samulski, R.J. Methods for the Construction and Propagation of Recombinant Adeno-Associated Virus Vectors. In "The Series Methods in Molecular Biology, Gene Therapy Protocols" Robbins, P. ed., In Press 1994.

14. Samulski, RJ AAV Vectors In Vivo In "Transgenic Animals - Generation and Use". Edited by Louis-Marie Houdebine. Harwood Academic Publisher. In Press January 1996.

ABSTRACTS

6. Xiao, X., Samulski, R.J. "The role of D sequence in AAV replication and virion packaging", American Society for Virology, Utah, June 1990.

7. Walsh, C.E., Liu, J.M., Young, N.S., Neinhuis, A.W., Samulski, R.J. "Gene Transfer and High Level Expression of a Human Gamma Globin Gene Mediated by a Novel Adeno-Associated Virus (AAV) Vector", August 1990.

8. Wei, Ohashi, Robbins, He, Kimak, Clark, Boggs, Fluharty, Gieselmann, Von Figura, Barranger, Samulski "Transfer and expression of the human glucocerebrosidase and arylsufatase A genes in bone marrow cells using a replication defective adeno-associated viral vector.", October 1991.

9. He, Wei, Ohashi, Robbins, Samulski, Boggs, Bahnson, Patrene, Clark, Kimak, Barranger "Transfer and expression of the human glucocerebrosidase gene", December 1991.

10. Walsh, C.E., Liu, J.M., Young, N.S., Neinhuis, A.W., Samulski, R.J. "Gene Transfer and High Level Expression of a Human Gamma Globin Gene Mediated by a Novel Adeno-Associated Virus (AAV) Vector. American Society of Hematology. 33rd Annual Meeting, 1991.

11. Brookens, M., Calmeils, T., Samulski, R.J., Meyrick, B., Gao, X., Huang, L., and Pitt, B.R. Adeno-associated virus vector for gene transfer to cultured ovine pulmonary microvascular endothelial cells. American Thoracic Society Meeting, 1991.

12. March, K.L., Hirshmann, J., Bauriedel, G., and Samulski, R.J. The adeno-associated virus as a gene transfer vector for human and non-human vascular smooth muscle cells. ASCR, 1992.

13. Walsh, C.E., Samulski, R.J., Young, N.S., Nienhuis, A.W, and Liu, J.M. Phenotypic correction of Fanconi anemia (FACC) in lymphoblasts and CD34+ progenitors with a recombinant adeno-associated virus (rAAV) vector. American Society of Hematology. 35th Annual Meeting, 1993.

14. Goodman S, Xiao X, Donahue RE, Moulton A, Miller J, Walsh C, Young NS, Samulski RJ, and Nienhuis AW. Recombinant adeno-associated virus mediated gene transfer into hematopoietic progenitor cells. Blood 80:167a, 1992..

15. J.L. Miller, R.E. Donahue, S.E. Sellers, R.J. Samulski, N.S. Young, A.W. Nienhuis. Recombinant Adeno-Associated Virus (rAAV) Mediated Expression of a Human γ-Globin Gene in Human Progenitor Derived Erythroid Cells. NHLBI, MD, UNC-CH, St. Jude's Children's Hospital, TN

16. Smith FI, Doll RF, Samulski RJ and Dyer CA. Gene delivery into brain cells using AAV vectors. Cold Spring Harbor Meeting on Gene Therapy, 1994.

17. Skulimowski, A., Hensley, L.L., Miller, C.H., Zhang, Y., Quattrocchi, K.B., Blight, A.R., Dull, S. T., Samulski, R.J. Viral Vectors for use in Gene Therapy of Malignant Gliomas. The American Association of Neurological Surgeons, 1995 Annual Meeting, April, Orlando, Fla.

IN PRESS

EXTERNAL ACTIVITIES

PATENTS

1.) **Title:** "AAV Transduction Vectors"
Serial No.: 5,139,941
Filing Date: 31 October 1985
Inventors: Dr. Nicholas Muzyczka, Dr. Paul Hermonat, Dr. Kenneth I. Berns, and Dr. Richard Jude Samulski.
Ownership: The University of Florida
Status: Licensed October 1992
Contact Person:
Dr. Susan Wray
Director for Patent, Copyright & Technology Licensing
Division of Sponsored Research
University of Florida
Gainseville FL 32611
(904) 392-8929
(904) 392-6600 FAX

2.) **Title:** "Helper-Free Stocks of Recombinant Adeno-Associated Virus"
Inventors: Dr. Thomas Shenk and Dr. Richard Jude Samulski
Ownership: DNX
Status: Pending
Contact Person:
Stephen Holtzman
DNX
303B
College Road East
Princeton, NJ 08540
(609) 520-0300
(609) 520-9864. FAX

3.) **Title:** "Adeno-Associated Virus Vector and CIS-acting Regulatory and Promoter Elements Capable of Expressing at Least One Gene and Method of Using the Same for Gene Therapy"
Serial No.: 07/923, 418
Filing Date: 31 July 1992
Inventors: Dr. Richard Jude Samulski, Dr. Arthur Nienhuis, Dr. Chris Walsh.
Ownership: University of Pittsburgh / NIH
Status: Continuation-in-Part Application filed 31 July 1992
Contact Person:
Frances Connell
Office of Intellectual Property
William Pitt Union
University of Pittsburgh
3959 Fifth Avenue
Pittsburgh PA 15260
Phone: (412) 648-2206

4.) **Title:** "Recombinant Viral Vector System"
Serial No.: 07/989,841
Filing Date: 4 December 1992
Inventors: Dr. Richard Jude Samulski, Dr. Xiao Xiao
Ownership: University of Pittsburgh / NIH
Status: Pending
Contact Person:
Frances Connell
Office of Intellectual Property
William Pitt Union
University of Pittsburgh
3959 Fifth Avenue
Pittsburgh PA 15260
Phone: (412) 648-2206

5.) **Title:** "Targeted Integration of AAV Vectors"
Inventor: Dr. Richard Jude Samulski
Ownership: University of Pittsburgh
Status: In Preparation
Contact Person:
Frances Connell
Office of Intellectual Property
William Pitt Union
University of Pittsburgh
3959 Fifth Avenue
Pittsburgh PA 15260
Phone: (412) 648-2206

Public Service

University of North Carolina-Chapel Hill and University Hospitals, "Mini Medical School", April 4, 1995. Lecture.

updated 9/15/97